COMPARISON OF GAHARU (*AQUILARIA MALACCENSIS*) ESSENTIAL OIL COMPOSITION BETWEEN EACH COUNTRY

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"I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree"

Signature :……..

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Date :…….

DEDICATION

Special dedication to my beloved father, mother, brothers and sisters……..

ACKNOWLEGMENT

In preparing this thesis, I was in contact with many people, researchers, academicians and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my beloved supervisor, Mr. Saifulnizam Tajuddin for encouragement, guidance, critics and friendship. I also indebted to FKKSA lecture for their guidance to complete this thesis. Without their continued support and interest, this thesis would not have been the same as presented here.

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ABSTRACT

Aquilaria is an evegreen tree growing up to 40 meters high and 60 centimetres in diameter. These trees frequently become infected with a fungus and begin to produce an aromatic resin commonly called Aloeswood, Agarwood and Oud. The resin is created in response to an attack from Phialophora parasitica, which is a parasite fungus or mold. The purpose of this project were to know the compounds in essential oil from gaharu that been produced by hydrodistillation, to analyse of the compound in the gaharu essential oil and to determine the quality, chemical compound and method use to extract the essential oil of gaharu between different origins. Chemical compound of an agarwood originating from agarwood (*Aquilaria* sp. probably *A. malaccensis*) were investigated by GC-MS. The differences in chemical composition between the agarwood in four difference countries are discussed. The samples are taken from gaharu production industry at Kelantan, China, India and Thailand. The extraction of gaharu essential oil also been done by using hydrodistillation. Firstly preparations of the sample were done by make the sample to the sawdust and after that soak it with water. Then setting the hydrodistillation set and heated up the sample that was soaked before. The temperature of the sample was maintained at 98^0C to 99^0C for three days. Collect the sample that diluted in solvent of ethyl acetate (EtOAc) and put it in sample bottle. Cleaned the sample from the water existed, before analyze it with GC-MS. The data from GC-MS were recorded in the understandable way. The compound between the four countries have large dissimilar, but it still some component is comparable between each country. The essential oil had been produced and been analyzed. If look from analysis result, component from oil sample of industry and laboratory were not identical

ABSTRAK

Aquilaria adalah pokok malar hijau yang hidup sepanjang 40 meter dan berdiameter 60 sentimeter. Pokok ini selalunya telah dijangkiti oleh fungus dan mula menghasilkan resin yang wangi dipanggil Aloeswood, Agarwood dan Oud. Resin ini dihasilkan untuk bertindakbalas dari serangan Phialophora parasitica, iaitu fungus parasit ataupun kulat. Tujuan projek ini adalah untuk mengetahui campuran minyak asal gaharu yang dihasilkan dengan menggunakan penyulingan hidro, untuk menganalisis sebatian minyak asal gaharu dan untuk menentukan kualiti, sebatian kimia dan cara yang digunakan untuk memerah minyak asal gaharu dari tempat yang berbeza. Sebatian kimia gaharu berasal dari *agarwood* (*Aquilaria* barangkali *A. Malaccensis)* telah disiasat dengan GC-MS. Perbezaan dalam komposisi kimia diantara gaharu dalam empat negara yang berbeza telah dibincangkan. Sampel telah diambil dari industri yang menghasilkan gaharu di Kelantan, China, India dan Thailand. Pemerahan minyak asal gaharu telah dibuat dengan menggunakan penyulingan hidro. Pertamanya, penyediaan sampel telah dibuat dengan menjadikan sampel kepada habuk kayu dan selepas itu proses merendam habuk kayu itu ke dalam air. Kemudian set penyulingan hidro disediakan dan panaskan sampel yang telah direndam sebelum ini. Suhu sampel telah dikekalkan pada suhu 98^0C hingga 99^0C selama 3hari. Sampel yang telah dilarutkan dalam pelarut dari *ethyl acetate* (EtOAc) dikutip dan dimasukkan ke dalam botol sampel. Sampel dibersihkan daripada sebarang kehadiran air, sebelum dianalisa dengan GC-MS. Maklumat dari GC-MS telah dicatatkan dalam cara yang difahami. Dari keputusan analisa, terdapat perbezaan yang besar diantara sebatian empat negara ini , tetapi masih ada sebahagian komponen yang sama diantara setiap negara. Minyak asal telah dihasilkan dan dianalisa. Jika dilihat dari keputusan analisa, komponen daripada sampel minyak dari industri dan makmal tidak serupa.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDIX

CHAPTER 1

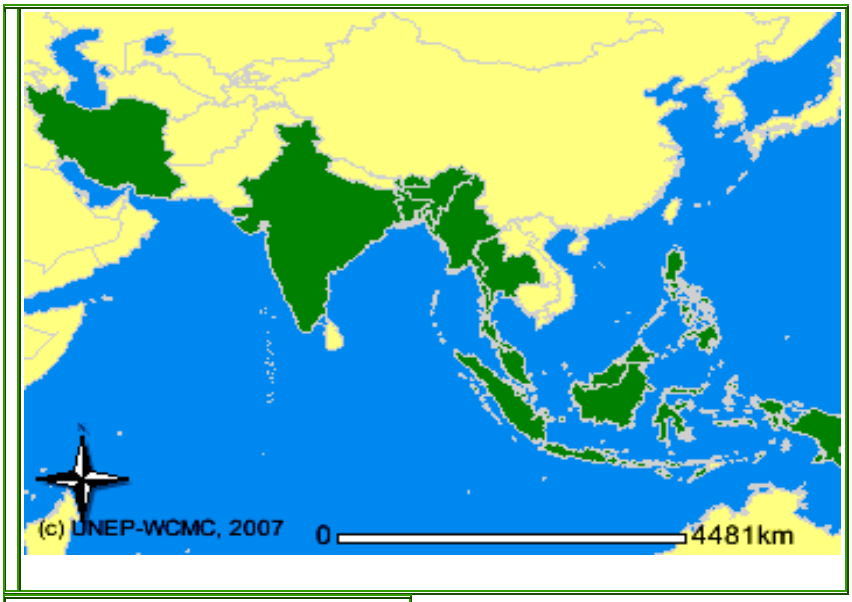
INTRODUCTION

1.0 Essential Oil

An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compounds extracted from plants. It may be produced by distillation, expression or solvent extraction. Essential oil is used in perfumery, aromatherapy, cosmetics, incense, medicine, household cleaning products and for flavouring food and drink. They are valuable commodities in the fragrance and food industries. Essential oil is also known as volatile oil and ethereal oil. It may also be referred to as “oil of” the raw plant material from which it was extracted, such as oil from clove. The term essential is intended to convey that the oil is an essence of the plant it is extracted from, and not in the more common sense of being indispensable, do not confuse them with essential fatty acids. Essential oil contains the true essence of the plant it was derive from. Essential oils are not the same as perfume oils or fragrance oils. Where essential oils are derived from the true plants, perfume and fragrance oils are artificially created fragrances, contain artificial substances or are diluted with carrier oils and do not offer the calibre of therapeutic benefits that essential oils offer.

Agar oil is distilled from the resinous portions of the wood of Aquilaria agallocha. This resinous wood is traded under the names “agar”, “aloe wood” or “eagle wood”. The tree occurs in patches in Bhutan’s southern hills, in Assam in India and in parts of West Bengal. Very little is known regarding why irregular portions of dark wood, highly charged with oleo-resin, appear in some trees but not in others, especially in and around old wounds and hollows. It is known that resinous infiltration occurs because of fungal attack, but the specific fungus responsible for the formation of agarwood has not yet been identified. Attempts to impregnate trees by driving pegs from trees already containing agar wood into trees not infected have not been successful. The distillation processes for agar oil consist of soaking agarwood in water for 60-70 hours. The wood is then disintegrated into powder in a chopper. The powdered wood, suspended in water to which 5 percent by weight of common salt has been added, is placed in a retort and heated over a furnace. The retort has a swan neck with a device for replenishing the water, without removing the lid during distillation. A Florentine flask made of glass or copper constitutes the receiver for the distillate. Distillation takes 30-32 hours. Because distillation takes place at atmospheric pressure, the process of total exhaustion of the wood is lengthy. The oil yield ranges from 0.75-2.5 percent of the wood.

Agarwood is a scented product obtained from a pathological condition of the wood of standing trees of certain Aquilaria species. Aquilaria is an evergreen tree growing up to 40 meters high and 60 centimetres in diameter. Aquilaria is native to Northern India, Laos, Cambodia, Malaysia, Indonesia, China and Vietnam as shown in (Figure 1.1). These trees frequently become infected with a fungus and begin to produce an aromatic resin commonly called Aloeswood, Agarwood and Oud. This resin is used by Traditional Chinese, Unanai, Ayuravedic and Tibetan physicians. This resin is created in response to an attack from Phialophora parasitica, which is a parasite fungus or mold. The fungus and decomposition process continues to generate a very rich and dark resin to form within its heartwood. The resin created as an immune response makes the most sacred oil on the planet.



Legend	
	Records of existence at country level.
	No distribution records at country level.

Figure 1.1 Distribution of Gaharu Across Asia Country

(Source: www.unep.org)

Aquilaria malaccensis is a species of plant in the Thymelaeaceae family. It is found in Bangladesh, Bhutan, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Singapore and Thailand. It is threatened by habitat loss (Barden, Angela, 2000). Aquilaria malaccensis is the major source of agarwood, resinous heartwood, used for perfume and incense (Broad, S., 1995). The resin is produced by the tree in response to infection by a parasitic ascomycetous mould, *Phaeoacremonium parasitica (*P. W. *et al.* 1996) and a dematiaceous (dark-walled) fungus.

The grade of agarwood essential oil is divided by 5 types, which are Grade Super A, A, B, C, and D. The Grade Super A is the most expensive compared to the others grade. The grade (and hence value) of agarwood and agarwood derivatives such as oil is determined by a complex set of factors including: country of origin; fragrance strength and longevity; wood density; product purity; resin content; colour; and size of the form traded. According to Heuveling van Beek and Phillips (1999), agarwood oil is graded based on the quality of raw materials, the method of distillation and the skill used in processing. It is said to be now virtually impossible to find pure agarwood oil (although a supposedly pure sample was received by TRP from a large international agarwood-trading group in Dubai). The grades are depends on the essential oil that can be extracted. Plant extracts as seen as a way of meeting the demanding requirement of the modern industry for the past two decades (Simandi *et al.*, 1996). Five basic odour classifications of agarwood incense aromas in Japan which is sweet, sour, hot, salty, and bitter (Morita, 1992).

Traders have quoted prices for pure agarwood oil as high as USD30 000/kg, such oil only being made to order. Grade-two oil costs approximately USD15 000/kg, but generally oil prices are between USD5000/kg and USD10 000/kg. However, cheaper oils, adulterated with perhaps a mixture of sandalwood and sesame seed oil, can be bought for a few hundred dollars per kilograms. Few traders nowadays, if any, can assess oil quality or purity and it is unlikely that there is much consistency between oil batches. Where agarwood prices have been obtained in currencies other than US dollars, they have been converted to that currency using the average interbank exchange rate for the period 1 January to 31 March 1999, based on rates provided by an on-line currency converter (OANDA, 2000). The following conversion rates were used: INR1 (Indian Rupee) = USD0.02353; IDR1 (Indonesian Rupiah) = USD0.00011; MYR1 (Malaysian Ringgit) = USD0.26311; THB1 (Thai Bhat) = USD0.02695. Agarwood powder is generally much less expensive than chips or flakes, with prices varying from around USD20-60/kg.

The first investigation on the chemical components of agarwood was reported by Kafuku and Ichikawa 1935 cited in (Shimada *et al., 1982*). Agarwood contains a sesquiterpene alcohol which produces its characteristic aroma. It was reported that 2-[2-(4'-methoxyphenyl) ethyl]chromone and 2(2-phenylethyl) chromone (Figure 1.2) (or flidersiachromone), through pyrolysis at 150ºC produces 4-methoxybenzaldehyde and benzaldehyde respectively (Hashimoto *et al.,* 1985). These molecules are odourless at room temperature but produce a long lasting fragrance upon burning.

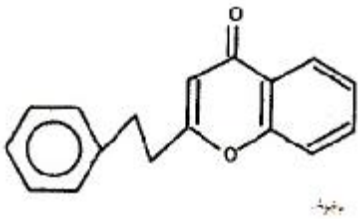


Figure 1.2 2(2-phenylethyl) Chromone

Form the chloroform-soluble fraction of the alcoholic extract of the alcoholic extract of A. agallocha, (Bhandari *et al.,* 1982) identified the molecules called aquillochin (a coumarinolignan), gmelofuran and agarol. Two chromones, known as chromone 1 (2-[2-(4'-methoxyphenyl)ethyl]chromone) and chromone 2(6-methoxy-2-[2-(4'-methoxyphenyl) ethyl]chromone), are components characteristic of "kanankoh" (in Japanese for the best quality agarwood from A. *agallocha*) and are either absent entirely or present only in small amounts in "jinkoh" (in Japanese for the lesser quality agarwood from other species of *Aquilaria*) (Hashimoto *et al*., 1985, Nakanishi *et al*., 1984,1986, Ishihara *et al*., 1991). Differences in other chemical components were also noted between the best and lesser quality agarwood (Table 1.1). According to the latest study of (Ishihara *et al.*, 1993a, 1993b), there are two types of kanankoh; one of them is rich in oxygenated guaiane and eudesmane derivatives, while the other contains oxo-agarospirol as a major sesquiterpene component. Similar chemical studies were conducted on agarwood from A. *agallocha* and other species of *Aquilaria* (Jain & Bhattacharyya 1959, Varma *et al*., 1965, Maheshwari *et al*., 1963a, 1963b, Barrett &

Buchi 1967, Yoneda *et al*., 1984). The results from a study by Yoneda and co-workers (1984) suggest that agarwoods of different origins may be distinguished chemically (Table 1.2).

Table 1.1: Differences in Chemical Component between Best and Lesser Quality Agarwood

Grade of agarwood	Compounds identified	Remark
Best	Sesquiterpenes: (-)-guaia-1(10),11-dien-15-al (-)-selina-3,11-dien-9-one (+)-selina-3,11-dien-9-ol	Absent from lesser quality agarwood
Lesser	Kusunol Dihydrokaranone Karanone Oxo-agarospirol	Present in considerable amounts

Table 1.2: Chemical Comparisons between Agarwood of Different Origins

Agarwood	Chemical components	Remark
Type A (*A. agallocha*)	Agarospirol Jinkoh-eromol Oxo-agarospirol α- and β-agarofuran Dihydroagarofuran Kesunol Nor-ketoagarofuran Dihydrokaranone	Abundant Not present in type B
Type B (*Aquilaria* spp.)	Agarospirol	

	Kusunol Jinkoh-eremol Oxo-agarospirol α-agarofuran (-)-10-epi-γ-eudesmol Jinkohol Jinkohol II	In large amounts, absent from type A

1.1 Problem Statement

Since *gaharu* is valuable, local entrepreneur has adopted water distillation technique that very much practice traditionally especially in rural areas of Cambodia and India (Chang *et al., 2002*). But now, local entrepreneur more prefer effective technique that produce higher yield of oil using hydrodistillation. Although several method of extracting essential oil is developed such as solvent extraction, expression and critical fluid extraction most are produce by hydrodistillation (Reverchon *et al., 1992*). Researchers are looking at various inducement techniques to produce aromatic *gaharu* on a commercial scale. The Forest Research Institute of Malaysia (FRIM) began researching in the late 1990s following a surge in market demand for *gaharu* and is still refining its inoculation technique. Based on anecdotes from Orang Asli collectors, researchers deliberately wound the tree trunk and indeed, *gaharu* was produced in varying degrees of formation, suggesting that it can be induced in standing Aquilaria trees by artificial means. But the grade obtained was inconsistent.

In *gaharu* producing species like Aquilaria, the tree will produce the resin to contain the infection from spreading, covering the wound and blackening the whitish heartwood. That's how *gaharu* is produce. "The challenge is to come out with high

quality or the desire grade and predictable volume to make planting a viable solution to over-harvesting of wild species," say Chang. But in Malaysia there is no instrument that can grade the *gaharu* essential oil base on the scientific ways. In Malaysia, most of the *gaharu* essential oil are been grading base on the experience, and it just from the physical look and its sense. So now there is still in the research about to grading the *gaharu* essential oil by using the technological methods.

1.1 Objective

- To know the compounds in essential oil from *gaharu* that been produced by hydrodistillation.
- To analyse of the compound in the *gaharu* essential oil.
- To determine the quality, chemical compound and method use to extract the essential oil from *gaharu* between different origins.

1.3 Scope of Study

In order to achieve the objective, the following scopes have been identified and to be applied:

- Study how to distil Malaysia *gaharu* by using hydrodistillation.
- Study the grade of *gaharu* essential oil that been produced in Malaysia and others country.
- Study the comparison *gaharu* compound among Thailand, China, India and Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.0 *Gaharu*

Agarwood or eaglewood is the most expensive wood in the world. It is valued in many cultures for its distinctive fragrance, and used extensively in incense and perfumes. Agarwood is the occasional product of two to four genera in the family ***Thymelaeaceae,*** with ***Aquilaria agallocha, Aquilaria crassna*** and ***Aquilaria malaccensis*** the best known species. The wood is formed as a result of the tree's immune response to fungal infection. The odor of agarwood is complex and pleasing, with few or no similar natural analogues. As a result, agarwood and its essential oil gained great cultural and religious significance in ancient civilization around the world. Agarwood , eaglewood , ***gaharu***, aloeswood are just a few of the name for the resinous, fragrant and highly valuable heartwood produced by ***Aquilaria malaccensis*** and other species of the ***Indomalesian*** tree genus ***Aquilaria***. The wealth of names for this dark and heavy wood (its Chinese name literally means 'wood that sinks') reflects its widespread and varied use over thousands of years.

2.0.1 Application of Agarwood

Agarwood has three principal uses, such as in medicine, perfume and incense. Smaller quantities are used for other purposes, such as carvings. These uses are described in more detail below.

2.0.1.1 Medicine

Agarwood has been used for medicinal purposes for thousands of years, and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicine, for example (Chakrabarty *et al.,* 1994; Fratkin, 1994). The Sahih Muslim, which dates back to approximately the eighth century, refers to the use of agarwood for the treatment of pleurisy and its use is referenced in the Ayurvedic medicinal text the Susruta Samhita. Agarwood is prescribed in traditional East Asian medicine to promote the flow of qi, relieve pain, arrest vomiting by warming the stomach, and to relieve asthma (Anon., 1995a). High-grade agarwood powder is prescribed in Chinese medicine (Yaacob, 1999) and is also used in the production of pharmaceutical tinctures (Heuveling van Beek and Phillips, 1999). (Burkill 1966) reported that Malaysians used agarwood mixed with coconut oil as a liniment, and also in a boiled concoction to treat rheumatism and other body pain. (Chakrabarty *et al.,* 1994) report that the often-discarded uninfected wood is used as *Kayu gaharu lemppong* by Malaysians to treat jaundice and body pains.

Bull 1930, cited in (Chakrabarty *et al.*, 1994) notes agarwood's use as a complex ointment for smallpox and for various abdominal complaints. Agarwood is also prescribed for dropsy, as a carminative, a stimulant, for heart palpitations, and as a tonic taken particularly during pregnancy, after childbirth and for diseases of female genital organs (Chakrabarty *et al.*, 1994).

2.0.1.2 Perfume

The use of agarwood for perfumery extends back several thousands of years, and is referenced, for example, in the Old Testament several times using the term 'aloes'. Both agarwood smoke and oil are customarily used as perfume in the Middle East (Chakrabarty *et al.*, 1994). In India, various grades of agarwood are distilled separately before blending to produce final 'attar'. Minyak attar is a water-based perfume containing agarwood oil, which is traditionally used by Muslims to lace prayer clothes (Yaacob, 1999). Agarwood perfumes are seldom pure agarwood oil, but instead use an alcoholic or non-alcoholic carrier, such as sandalwood oil. The cheapest agarwood perfumes are either synthetic or a blend of oils, each with different qualities and fragrances. Although there are several commercially available synthetic agarwood fragrance compounds, they can produce only low-quality agarwood fragrances, owing to the chemical structure of natural oil (Heuveling van Beek and Phillips, 1999). Agarwood essences have recently been used as a fragrance in soaps and shampoos (Kadir *et al.*, 1997), cited in Schippmann, 1999. Agarwood is said to have been highly prized by European perfumers in the mid-1990s (cited in Chakrabarty *et al.*, 1994).

2.0.1.3 Incense

Agarwood incense is burned to produce a pleasant aroma, its use ranging from a general perfume to an element of important religious occasions. Irregular chunks of agarwood, usually a few centimetres long and weighing 10-200 g, may be cut or broken into smaller pieces and then burned, usually in a specially made incense burner (Heuveling van Beek and Phillips, 1999). Agarwood powder and dust cannot be burned directly in incense holders, but can be used to make incense sticks or coils for indoor fragrance, and are used for religious purposes by Muslims, Buddhists and Hindus (Yaacob, 1999).

Taiwanese consumers purchase agarwood for the manufacture of incense sticks, which are used in Agarwood perfume, chips and powder in Malaysia, 1999 Agarwood incense sticks on display in Taiwan, 1998 prayers during many traditional festivals and ceremonies to bring safety and good luck (TRAFFIC East Asia-Taipei, in litt. to TRAFFIC International, 2 May 2000). Both Indians and Chinese have used agarwood

as an essential ingredient of incense sticks in the past, but in the present day incense sticks generally do not contain agarwood, although Indian traders report that high-quality Indian incense sticks destined for export may have a drop of agarwood oil added to them (Chakrabarty *et al.,* 1994). Agarbattis are incense cones, which also originally contained agarwood powder but seldom do so now because of the high price of agarwood. Instead, the light cream/brown powdery waste material obtained from oil distillation (with little or no resin content) is used to provide a basic carrier for other, cheaper, fragrant ingredients. This waste agarwood powder sells for around USD5/kg.

Japanese incense products are very different, with most of the highest-grade products made using natural raw materials which include ground agarwood extracts combined with other ingredients such as sandalwood and benzoin and then carefully molded and baked. Pure agarwood is also burned as incense in Japan. The user breaks pieces off and burns small pieces as required, hence large sections of wood will last several years (Heuveling van Beek and Phillips, 1999). In Japan, a revival in the ancient art of Koh doh, the incense ceremony, has revitalised interest in agarwood (Katz, 1996).

In Malaysia, Muslims burn agarwood splinters or chips to produce incense during special religious occasions, particularly at gatherings, and agarwood incense has been recorded in use there during Ramadan prayers (Chakrabarty *et al.,* 1994). Some Malay tribes fumigate paddy fields with agarwood smoke to appease local spirits (Chakrabarty *et al.,* 1994). Agarwood incense is used for various purposes in the Middle East, especially during prayers (Yaacob, 1999). Agarwood chips and splinters are also burned in bathrooms and incense is used as a customary perfume. Party hosts place agarwood chips over hot charcoals, the aroma signifying the end of a party.

2.0.1.4 Other Application

Burkill (1966) reported that grated agarwood has been used in Malaysia for cosmetic purposes, particularly during sickness and after childbirth. The use of agarwood bark as a writing material has also been documented extensively and agarwood is used for chronicles of important and sacred religious books. Use as a substitute for paper is also known from the mountaineers of Annam (Vietnam) and from China (Chakrabarty *et al.*, 1994). Twine is reported to be made from Aquilaria in Malacca (a province of Malaysia) (Chakrabarty *et al.*, 1994).

Although it may be possible to use healthy Aquilariawood to make simple ornamental boxes, this wood is typically too light and fibrous (rather like balsa wood) to be suitable for furniture, construction or even carving. Some foresters in India have suggested using Aquilaria wood for constructing tea-boxes (Chakrabarty *et al.*, 1994). Aquilaria bark was reportedly used for this purpose during the nineteenth century (Heuveling van Beek and Phillips, 1999). There are a considerable number of craft shops offering religious ‘agarwood’ sculptures, usually Bhuddhist figures. Although a proportion of immature agarwood is used in this trade, most statues are not made with agarwood, owing to its soft and flaky properties, which make it unsuitable for carving. Instead, tropical hardwoods are treated to resemble agarwood. The wood is blackened by injecting oil or tar into tree trunks and may also be impregnated with agarwood perfume (Heuveling van Beek and Phillips, 1999).

Agarwood is used to produce statues and religious objects (e.g. statues of Buddha) in Taiwan (TRAFFIC East Asia-Taipei, in litt. to TRAFFIC International,